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EXPLOSIVES DETECTION SYSTEMS EMPLOYING BEHAVIORALLY MODIFIED RATS AS SENSORY ELEMENTS

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I. INTRODUCTION

Synopsis of the Experiment. The research program was concerned with improving the operational reliability of animals employed as the sensory element in explosives detection systems. Four basic theses were investigated and the research results in each of these areas represented an original contribution to the developing field of biosensor detection systems. The theses are: (A) It is postulated that rats can detect, via their olfactory sensory function, some component of the military explosive 2, 4, 6-trinitrotoluene (TNT) in minute quantities, (B) It is postulated that if A above is true, then proper behavioral conditioning can cause the animals to operantly signal their awareness of the presence of some effluent of TNT in the ambient air, (C) It is postulated that if B is true, then it should be possible to train several subjects simultaneously using a semi-automatic test station employing operant and classical conditioning paradigms, (D) It is postulated that if B is true, then it should be possible to classically condition the test subjects to anticipate the reward that was heretofore forthcoming only as a consequence of an operant. The state of cerebral arousal resulting from the anticipation should then result in an anticipatory evoked spectral change (AESC) in the cortical electroencephalogram (EEG) of subjects so conditioned.

The research protocol spanned several disciplines including physiology, behavioral science, electronics engineering, biochemistry and explosives technology. Unfortunately, space limitation precludes significant discussion of the relative merits of Physico-Chemical, Electromagnetic (the existing explosives detecting techniques), and Biosensor systems. This limit also narrows the discussion of the overall experiment to a rather short discussion of the results. The matters of subject selection, behavioral training and instrumentation are amply discussed in MERADCOM Report 2343 dated December 1981, entitled "Investigation of Behaviorally Modified

Rats for Use in Explosives Detection Systems" and in the author's doctoral dissertation (same title) dated July 1981 GW University, Washington, DC and these matters will not be treated in any depth here.

It must suffice to state that the test subjects were chosen to be small, inexpensive, non-controversial, clonelike mammals which manifested macrosmatic characteristics and which evidenced no emotional dependence upon the human race. The subject most closely meeting these specifications was a strain of albino rats (Sprague-Dawley) bred for laboratory use over many generations. For greatest unit-to-unit similarity, only male rats were used and then only then whose body weight was 500± 100 grams.

Experiments with dogs at MERADCOM and elsewhere revealed that the detection performance of animals which are simply behaviorally modified and then sustintatively rewarded varies hourly and diurnally and thus the user is never really certain that the ultimate performance is being extracted from the animal in any given search. Further there is absolutely no reason to believe that these animals are always willing or even able to perform at maximum sensitivity and specificity.

It was desirable therefore to devise a scheme whereby an animal biosensor could be induced to sense target substances with unprecedented singleness of purpose, while at the same time maximally exercising its natural ability to discriminate the desired target substance in the presence of extremely heavy clutter.

Thus, the four postulates and the experimental program were devised to demonstrate that a reliable biosensor detector could be reduced to practice in a variety of Army and civilian explosives detection scenarios.

II. EXPERIMENTAL PROTOCOL

Anticipation is the basis of conditioned behavior regardless of whether the subject anticipates a pleasant or aversive result from a known pattern of events. The effectiveness of any conditioning paradigm is directly related to the intensity of anticipation and thus one must seek that rewarding stimulus which most intensely effects a particular class of experimental subjects for the longest period of time. The anticipation effect exists, at least in all mammals, regardless of whether the behavioral conditioning regimen is based upon operant (instrumental) or classical (Pavlovian) principles.

It is not possible in this short paper to discuss the details of operant and classical conditioning but these matters are well known and exhaustively treated in the vast literature of behavioral science. It is sufficient here to report that, in this research, an operant regimen was established to determine the "conditionability" of the subjects and to

establish for the subject the relationship between the presence of TNT vapor (the olfactory stimulus) and the reward stimulus. Following this initial training phase, the subject was classically conditioned in order to maximize the anticipation of reward and to eliminate the need for any operant response. This approach rendered the subject to the status of a biosensor detector element in a system where only intense anticipation was required of the subject. Since this is essentially a sub-conscious response to the initiating stimulus (TNT Vapor) there is no opportunity for the subject to willfully modify this response to the odorant and, in essence, a subject operating in this mode "cannot tell a lie" to gain a reward and it cannot refuse to annunciate detection of the target substance (as dogs are known to do on occasion).

The ultimate rewarding stimulus for rats was defined by Dr. James Olds in the 1950's as electrical stimulation of the Medial Forebrain Bundle (MFB) in either cerebral hemisphere. Consequently, no research time was expended in investigating this well established fact and all subjects surgically received a chronic electrode implant in this brain area. The reward stimulus was then termed electrical brain stimulus or EBS. Because of the evident pleasure manifested by all subjects so stimulated, Olds called the MFB the "pleasure center" and this terminology is still in use.

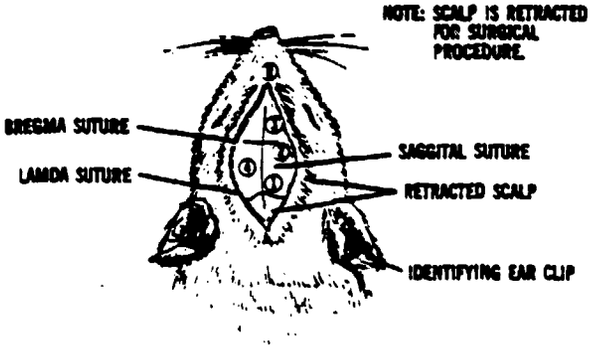
It should be noted that EBS results in no change in homeostasis as is the case with sustentative rewards such as food or water and thus the sensorium is much more constant than that of nutritionally deprived subjects. Even more important is the fact that EBS does not satiate as do sustentative rewards and thus anticipation remains constant even in long (>2hours) search sessions. This factor alone is sufficient to demand EBS as a reward stimulus!

Thus, the training paradigm prepared the subject to intensely anticipate an extraordinary pleasure whenever he detected the odor of TNT. However, the final test procedure allowed for no EBS during TNT stimulus runs since 100% reward tends to result in habituation which ultimately dulls anticipation and thereby actually lessens the probability of detection in a biosensor system of this nature. In an operational detection scenario using a rat as the sensor element, EBS would be forthcoming on a random basis in 70% of those events where an AESC was evident since, as described later, this percentage of reward resulted in maximal anticipation which, I believe, in turn resulted in maximum AESC which then gave optimal probability of detection.

Electrode Sites. The EBS electrode site was defined a priori but the definition of the electroencephalograph (EEG) electrode sites required some effort. First, the electrode had to be large (> 1 mm^2) since no specific locus for the AESC would be surmised. An epidural location seemed most appropriate since this tissue is quite rugged whereas the gross electrodes

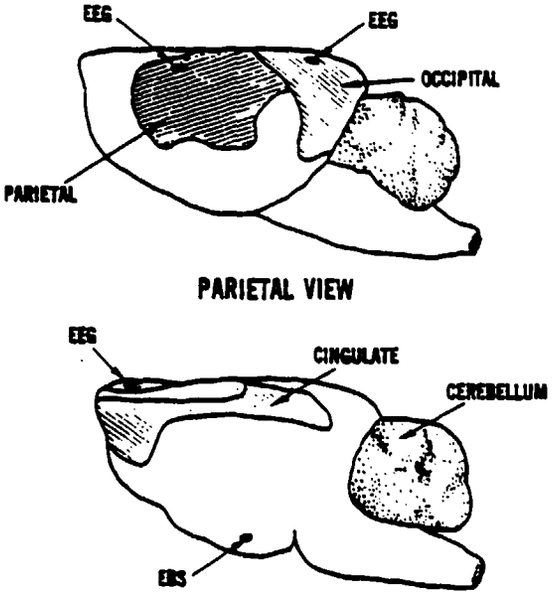
would surely cause avulsion and necrosis in direct contact with cortical tissue. Thus the matter of penetration was resolved and the EEG electrodes (0-80 stainless steel screws) were threaded through the skull and juxtaposed to the dura mater. Since the limbic system must at least madiate if not originate the pleasure reaction, a locus proximal to the cingulate gyrus was chosen as the principal electrode site. A parietal electrode was implanted to acquire signals from the associative areas. Figs 1 and 2 show these loci.

Stimulus Delivery. Fig 3 depicts the system used throughout the program. The subject was placed in severe restraint (to minimize motor signal artifacts in the EEG) in a copper-shielded enclosure -- a Faraday Box -- which also contained the EEG preamplifiers (PAR Model 113). Odorants were delivered in two classes: TNT and Neutral. Neutral stimuli were random selections of asphalt, cedar and pine. The odorant delivery system used "zero" air to maintain a constant concentration of odorant from day to day but the ambient air in the Faraday Box was simply laboratory air. The Faraday Box was operated at a slight negative pressure so that TNT build-up did not occur in the proximal to the subject. The effluents were exhausted external to the laboratory and the subjects gave no evidence - ever - of awareness of TNT contamination of the laboratory. Constant cleanliness was essential and heroic efforts were used to assure continuing freedom from TNT build-up in any of the laboratory or domiciliary areas. The negative pressure source also assured a rapid lowering of ambient odorant concentration during non-stimulus periods between odorant presentations.



EEG & EBS ELECTRODE SITES (TREPHINE LOCATIONS)

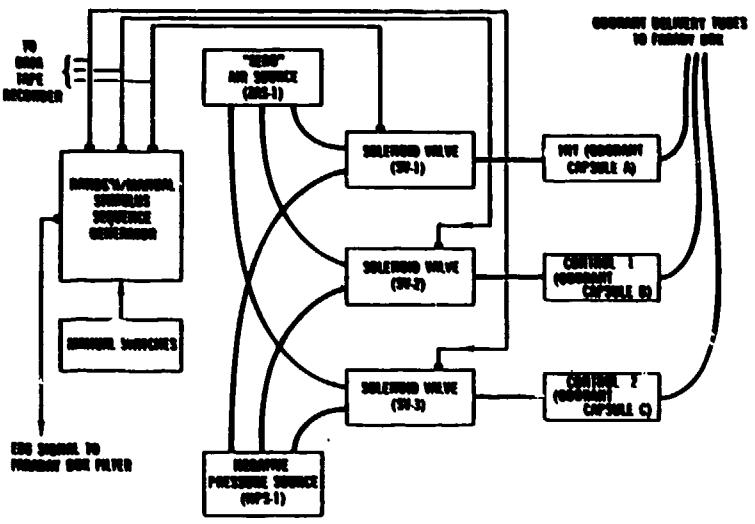
FIGURE 1



PARIETAL & SAGGITAL VIEWS OF EEG & EBS ELECTRODE SITES

FIGURE 2

All stimuli - including EBS - could be either randomly or manually sequenced. During most test runs the sequencing was manual so that even in short runs approximately the same number of TNT and Neutral stimuli were delivered, whereas long conditioning test runs were generally accomplished by use of the automatic random sequencer.

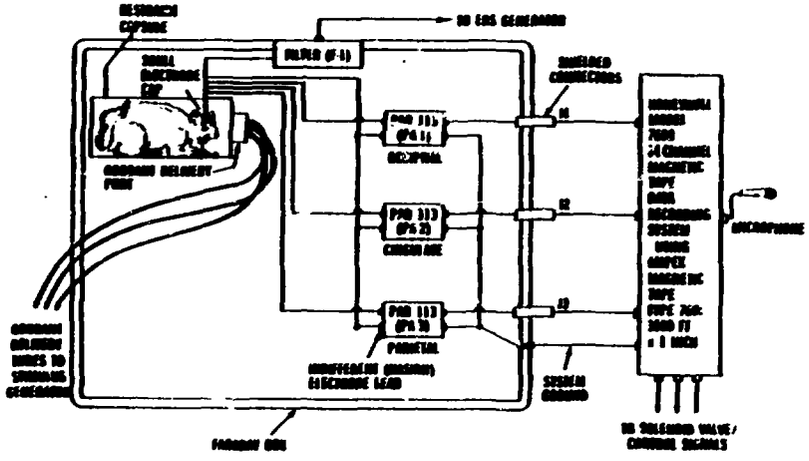


EBS & ODORANT DELIVERY SYSTEMS
FIGURE 3

No attempt was made to quantify the TNT molecular concentration due to the extreme difficulty of such measurements even in analytical chemistry laboratories. In a simple physiology laboratory the task was determined to be virtually impossible. Also, knowledge of exact concentration of odorants was not necessary to the research program. Ultimately, sensitivity must be quantified relative to dogs but a dual dog/rat program was out of the question at all times during the research period.

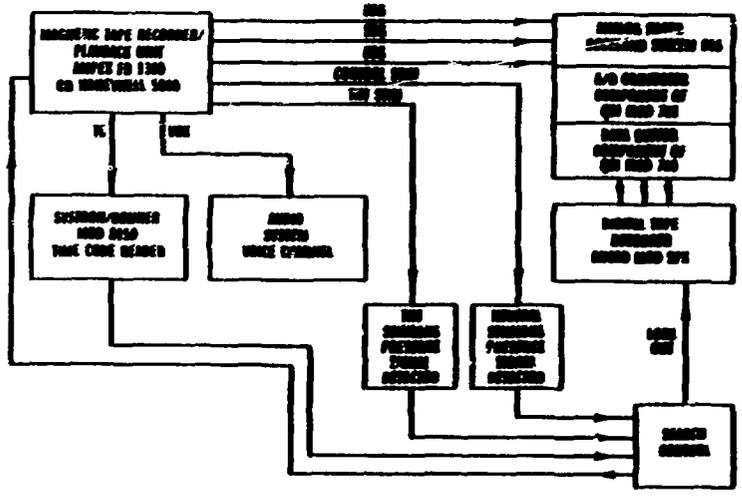
Data Systems. The data recording system is depicted in Fig 4 and the data playback system is shown in Fig 5. The PAR EEG preamplifiers were set to 6 DB bandwidth points of 0.3H_z and 300 H_z with gain set at 10,000. Thus, the EEG signals (which were found to range between 20 and 150 microvolts at the dura mater) were delivered at levels of 200 to 1000 millivolts to the analog magnetic tape recorder (Honeywell Mod 7600). In addition to EEG signals, the recorder also acquired an IRIG time code, voice commentary and the control signals from the odorant delivery solenoid valves in the stimulus delivery system. The solenoid control signals were of extreme importance in the final data analysis programs, since timing of odorant bolus arrival in the Faraday Box was thereby effected. Data recording was usually continuous in 2 to 3 hour segments so that a great mass of "resting" background EEG data were available if needed.

The digitization of the analog EEG data was carried out with an analog system bandwidth of DC to 312 Hz (IRIG Low-Band), digital system bandwidth of DC to 200 Hz with 512 data points per second and a density of 1600 bits per inch.



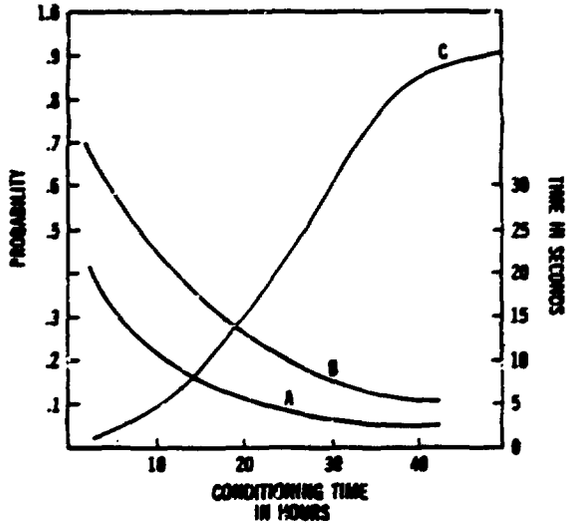
DATA RECORDING SYSTEM

FIGURE 4



DATA PROCESSING SYSTEM

FIGURE 5



A = TREADLE PRESS TIME FOLLOWING TERMINATION OF ODORANT FLOW
B = PROBABILITY OF FALSE RESPONSE
C = PROBABILITY OF TRUE RESPONSE

DAY PERFORMANCE VS CONDITIONING TIME

FIGURE 6

III. RESULTS

Verification of Theses A, B & C. Prior to this research effort, there were no known data which could unequivocally demonstrate that rats were able to detect TNT, even though assumptions were voiced to the effect that this capability should exist. It was essential, then, that Thesis A be proven valid at the earliest possible moment in the history of the research and it became apparent in the formulation of the experiment that Thesis A was most convincingly proved by the concurrent proof of Thesis B.

A total of 818 3-second data epochs were extracted from a total population of 1300 similar epochs for the purpose of final data analysis (the unused 482 epochs were contaminated by excessive noise or motor artifacts in early data runs). Spectral analysis of 100 epochs revealed that the experimental 312 Hz recording bandwidth was quite adequate. Indeed, the data evidenced little activity in any epoch above 100 Hz and the average data epoch revealed an EEG with maximum spectral density occurring in human EEG's.

Fig 6 summarizes the results of the behavioral analyses of the data mass. It was soon apparent that the conditioning regimen was quite effective as curve "C" demonstrates. Forty hours past the state of total naivity, the average subject would annunciate by treadle press (in the first or operant phase of conditioning) his awareness of the presence of the target substance TNT. At the same training landmark, the subjects evidenced a greatly diminished tendency to falsely signal TNT presence.

Concurrent with the diminishment of false annunciation was the decrease in the tendency to attempt to prolong the pleasure of EBS by treadle pressing after the disappearance of TNT-laden air from the stimulus delivery tubes (Curve A). This factor is important since the low probability of continued treadle pressing at 40 hours of training implies that the subject has clearly recognized that EBS is absolutely related to TNT presence and that EBS is not available in any other case. The early high rates of signalling in the absence of TNT was most probably due to the intense desire for EBS of "no reward without TNT odor" since diminishment of this activity occurred as subject "awareness" developed.

As noted earlier, some extinguishment of behavioral conditioning invariably accompanies any operational protocol in which a conditioned subject is rewarded each time a correct operant or classical response is effected in response to stimulus. Such behavioral response (habituation) can seriously alter the course of an experiment, since these effects will probably be of indeterminate magnitude a posteriori, thus leaving an unknown degree of bias in the overall experimental results. To preclude this untoward eventuality, fifteen rats were studied for the effects of unity EBS/odorant presence ratio. Habituation was generally evident in those

subjects which had passed the 40-hour point in their conditioning regimen (during which time a unity ratio was the rule) by about 20 hours. Although the diminishment of the detection performance -- as evidenced at this stage of training by treadle press -- was highly variable between subjects, the average diminishment observed in all fifteen subjects was found to be a drop to about 0.7 probability of detection. Since all the selected subjects had evidenced 0.9 or better probability of detection near the end of their initial 40-hour conditioning regimen this diminishment of performance was quite significant from the standpoint of practical application in explosives detection service.

As the EBS/odorant presence ratio was varied from unity to 0.3 by withholding EBS in a pseudo-random manner, the average subject began initially to increase his probability of detection (due to enhanced anticipation) and then extinguishment again appeared. The curve of Fig 7 represents the average performance of the test subjects.

It is important to stress that the exact nature of the olfactory cue was never a matter for investigation in this research. Certainly military grade TNT contains several substances in addition to 2,4,6-trinitrotoluene, but these substances are always present in approximately the same proportions in all samples of military grade TNT, and thus it is of no consequence to this research if the animal identifies one component or all components of this explosive, since detection of the aggregate substance is the desired experimental goal. Further, the matter of distractants was not pertinent, and hence this avenue of investigation was not explored in this research.

Similarly, no extensive effort was made to find the "ultimate" neutral stimuli since, in effect, <u>any</u> olfactory stimulus other than TNT was, by definition, neutral, and degrees of neutrality were of no interest. In the long term, the matter of distractants (or non-neutral, non-desired) stimuli will be of interest to the overall program of the sponsor, but such investigations were totally beyond the scope and intent of the instant program.

At the end of a forty-hour test sequence, the average subject clearly verified Theses A & B by his behavior. To summarize: In the absence of TNT vapor, the subject generally ignored the treadle after about thirty hours of conditioning, except for an occasional random press (perhaps this represented an attempt to hasten EBS, or just "to see if anything good will happen"). When TNT vapor was present at the 40 hour point, the animal began furiously to press the treadle in an attempt to extract the maximal pleasure from what it may have come to recognize as an ephemeral event.

Fig 6 graphically demonstrates the validity of Theses A & B and these early results demanded that the program continue in an attempt to prove the very important Thesis D.

The learning patterns, while exhibiting variations due to the ever-present physiological and psychological differences in subjects, were, overall, similar to the general trends shown in Fig 6, and at the end of the 40-hour training periods, all subjects were performing as the curves depict within limits of about ±30%.

Thesis C was proved in achieving the proof of Theses A & B by the use of a group of five behaviorally shaped subjects which were totally naive to the EBS/TNT relationship at the start of testing. Each test subject was given identical TNT/EBS pairings, thus simulating sequentially what would have occurred simultaneously had sufficient laboratory facilities been available.

It is pertinent to note here that the continuously observed indifference evidenced by all test subjects toward man en masse or as an individual argues strongly in favor of the concept of automatic training, since there would be no resulting impact upon the depth of conditioning even if the test operators were changed several times during training.

This final factor was of great significance not only to the immediate test results, but in consideration of future systems employing rats as biosensor detector elements, since the optimal biosensor animal must be one which requires no emotional ties to any human element employed in overall system operation.

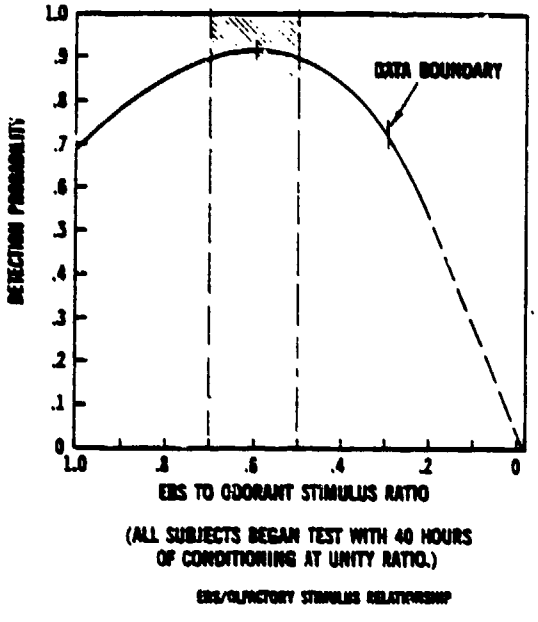


FIGURE 7

Verification of Thesis D

One of the two successful signal analysis - or feature extraction - techniques used for the proof of Thesis D was a metric in the general form of the Covariance process. The covariance process will not be described here since it is well defined in the literature. It is necessary to note that the covariance of the raw data was first examined with a scanning window width of 11 data points and then these covariances were in turn examined with a window width of 31 data points. Thus, the final covariance data used in the proof of Thesis D was the "covariance of the covariance".

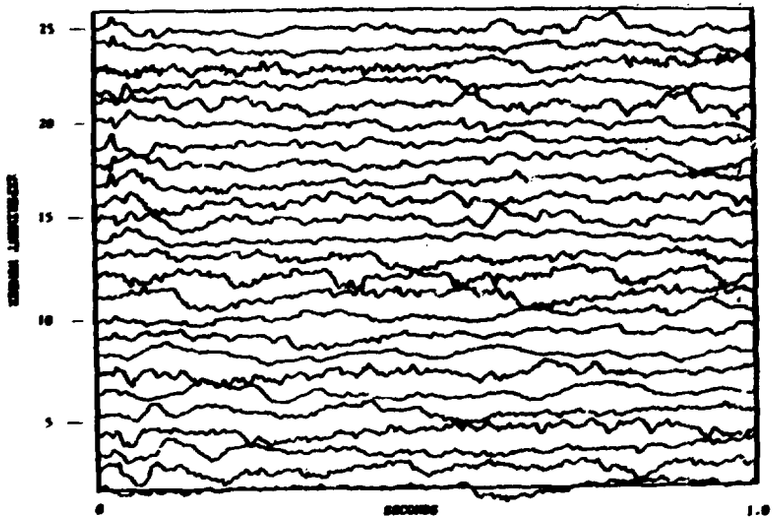


FIGURE 8

25 MONOPOLAR RAW DATA EPOCHS FOR INTERVAL T_ϕ FOR RAT C.

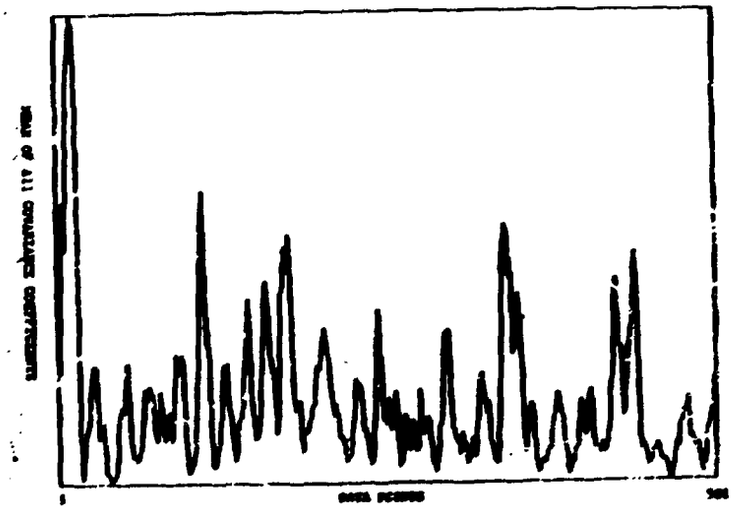


FIGURE 9

MEAN OF Δ11 COVARIANCE COEFFICIENTS FOR INTERVAL T_ϕ FOR 25 DATA EPOCHS RAT C.

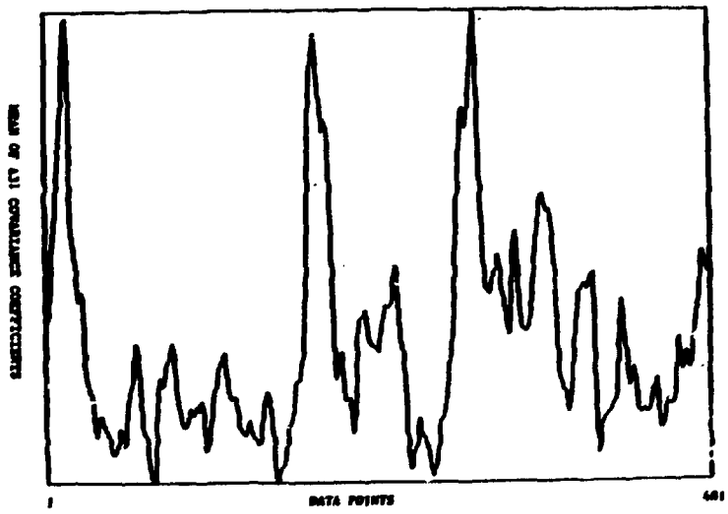


FIGURE 10

Δ31 COVARIANCE MEAN OF Δ11 COVARIANCE MEANS OF INDIVIDUAL RATS DURING INTERVAL T_ϕ.

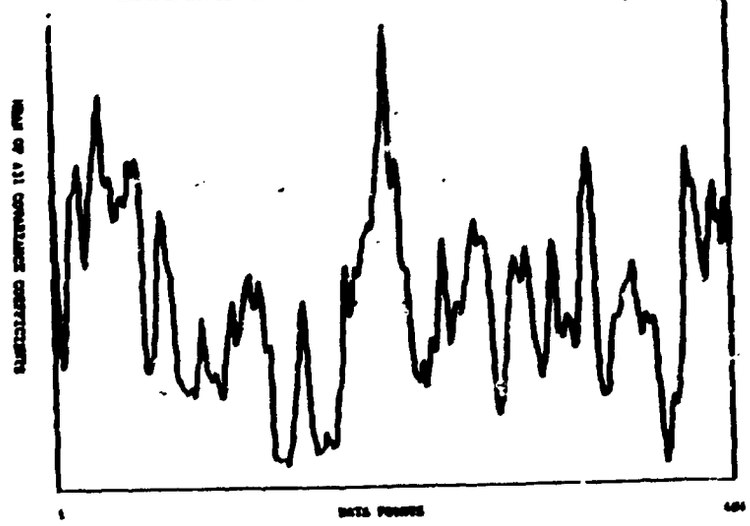


FIGURE 11

Δ31 COVARIANCE MEAN OF Δ11 COVARIANCE MEANS OF INDIVIDUAL RATS DURING INTERVAL T_ϕ

The final form of the data epochs subjected to analysis consisted of three one-second intervals defined as follows: FN

a. Interval T - 1: The period of data occurring in the one-second interval immediately prior to the trigger.

b. Interval Tø: The period of data occurring in the one-second interval immediately subsequent to the trigger.

c. Interval T + 1: The period of data occurring in the one-second interval immediately subsequent to the Tø interval.

d. Similarily N-1, Nø, and N+1 represent the same time intervals for the case of neutral stimuli.

With these terms in mind, it will now be of interest to examine Figs 8, 9, & 10. Fig 8 depicts a small segment of the raw analog monopolar signals obtained from the cingulate electrode of Rat C. Here are presented twenty-five epochs of the interval Tø with the relative amplitudes of the signals maintained in true relationship to the original signals.

Fig 9 is a plot of the Δ11 covariance coefficients versus time (in data points/second) for the period covering the first second subsequent to a TNT odorant bolus release. The first large peak (about data point 22) occurred at about 40 milliseconds subsequent to the activation of the TNT odorant solenoid valve relay. During initial data analysis this event was assumed to be perhaps fortuitous, but after examination of similar data epochs across the rat population, it became apparent that this peak was related to the solenoid valve relay activation in a relatively invariant manner. Such a repetitive event was seen to exist also in all Nø plots (where the neutral stimulus odorant-delivery solenoid activation pulses were used to define the time period of analysis. The most reasonable explanation for this ubiquitous signal feature is that it represents the effect of the relay "click" on the auditory processing circuits in the rat brain!

Fig 9 and similar plots for other epoch sets shows some evidence of olfactory stimulus recognition, (if one knows exactly where to look) but, standing alone, this set of plots would not suffice for verification of Thesis D.

FN The trigger, as noted earlier, is defined as the leading edge of the DC pulse applied as the activation signal to the odorant-delivery solenoid valve relays. Δ11 is the symbol used to designate those covariance processes in which the window width was 11 data points and Δ31 is the symbol used to designate those covariance processes in which the window width was 31 data points.

Since the transit time of the odorant bolus was known to be 500 ± 70 milliseconds, a careful examination of the mid-abcissa region of the T∅ plot in Fig 9 may reveal some evidence of unusual covariance at about data point 220, but this feature is not as immediately apparent as one might wish and, in order to make the feature at data point 220 more evident, a second application of the covariance process was effected on all Δ11 data sets using a window width of 31 data points.

Fig 10 is a plot of the results of this process. This plot is of great significance since it represents graphical evidence of the validity of Thesis D!

To recapitulate the specific events displayed in Fig 10:

a. 87 separate experiments derived from the data for Rats A, B, C, D, 98, 100 and 103 were analyzed over the T∅ interval.

b. Δ11 covariance means were obtained for the ensemble of 87 experiments.

c. Δ31 covariance means were derived for the summed results of the Δ11 calculations.

d. The unique features seen in Fig 10 were obtained only for TNT stimuli.

Fig 11 is the neutral olfactory stimulus equivalent to Fig 10 in the N∅ interval. One may observe the clearly evident auditory signal near data point 24 in the N∅ interval. However, there is no significant pairing such as that seen in Fig 10.

In Fig 11, the earliest N∅ peak which could possibly be related to an olfactory stimulus occurs at about data point 290 which represents a time beyond the upper measured limit of odorant arrival time. Thus, while the single large peak near the center of N∅ ensemble may or not represent some olfactory related event, there is little doubt that the twin peaks seen in Fig 10 are unique evidence of an event related only to the presence of TNT vapor.

Based upon the foregoing analysis, it is virtually certain that a unique feature appears in the output of the Covariance Process when -- and only when -- the input EEG is derived from a fully conditioned test subject during a period of olfactory stimulation by TNT vapor.

The Segmentation Process. This metric is fully described in the author's report (MERADCOM 2343) and in his doctoral dissertation. Basically this metric places a time scanning iterative process into the

category of an adaptive feature extraction technique.

Fig 12 depicts the result of application of the Segmentation Process to a single TNT stimulus experiment from the data set for Rat B. In this and similar plots the scanning window width was set - as it was throughout the final iteration of this process - at 128 data points, and the value of β (a reference threshold) was set by trial to increase the fine structure of the plot while maintaining relative freedom from random event peaks. Note that the abcissa is the time axis, and, in terms of the Covariance Process terminology, intervals T - 1, TØ and T + 1 are contiguous in presentation in this figure. Since there is a 500 ± 70 millisecond delay between the solenoid release signal -- which occurs in this figure at 1.0 on the Time axis -- and the arrival of the odorant bolus, those events which occur at or near 1.5 on the Time axis are worthy of close scrutiny.

Note the double peak near time 1.5 in Fig 12. Here β has been lowered to the experimentally determined limit of 0.175.

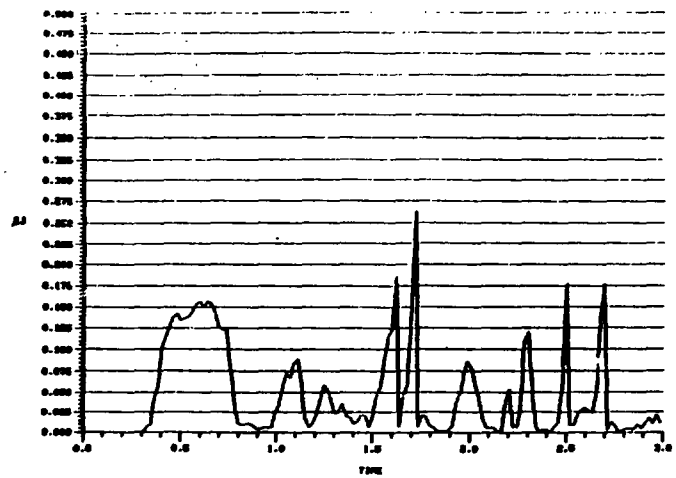
The similarity to the plot of Fig 10 is interesting since the same general result was obtained from two greatly different metrics applied to two different data sets.

In Fig 13, the Segmentation Process output is subjected to spectral analysis which elucidates two dominant peaks and one apparently spurious peak. The significant peak at 8 Hz may possibly represent those physiological processes associated with the so-called Alpha rhythms in mammals (8 to 13 Hz) or it may have a more obscure meaning here. The peak at 20 Hz occurred in one subject only and may be an artifact which would be insignificant in a larger data sample. The most significant grouping of peaks in this figure is that existing between about 10 Hz and 17 Hz.

Fig 14 also manifests a dominant peak at about 7 to 8 Hz, and it probably has the same significance as it does with TNT data plots. A secondary broad peak centered at about 10 Hz is seen as well as very small peaks out to 125 Hz.

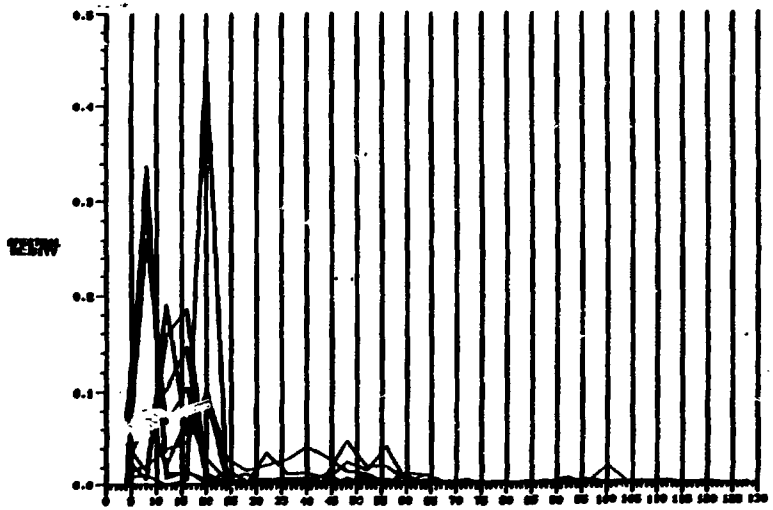
Clearly, the AESC appears in the plots of Fig 13 as the frequency/density peak seen between the 8 Hz cluster and the single 20 Hz peak. The composite structure is so different from that generated by the neutral stimulus data presented in Fig 14 that the peak cluster in Fig 13 must be called unique.

This, then, is the final argument in support of the postulate of Thesis D. Two totally different processes -- Covariance and Segmentation -- present strong evidence of the presence of unique cortical spectral features occurring in close time coincidence with the application of TNT



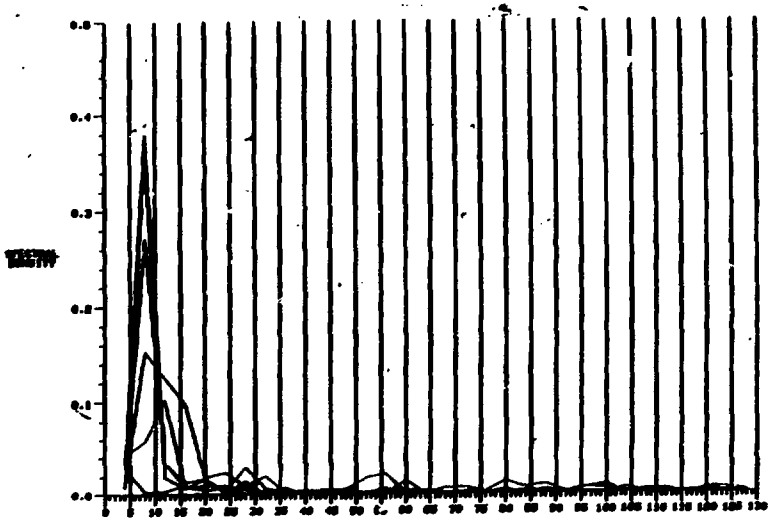
ERROR FUNCTION VERSUS TIME IN DATA EPOCH FOR β = 0.176, TNT STIMULUS.

FIGURE 12



COMPOSITE SPECTRAL DENSITY FOR ALL RATS, TNT STIMULUS.

FIGURE 13



COMPOSITE SPECTRAL DENSITY FOR ALL RATS, NEUTRAL STIMULUS.

FIGURE 14

olfactory stimuli in the specially trained test population. Taken individually, each process presents a strong case for the occurrence of the AESC; taken collectively, there is little doubt that an identifiable feature does exist as a direct consequence of the TNT stimulus, and there is little doubt that this feature can be identified by existing microprocessor technology, especially if three or more experiments can be summed before recognition is demanded by the detection system operating protocol. It appears therefore, that Thesis D is proved to be valid.